



5fpo-02-34 amended sequence listing

<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 26666U

<150> KR 2002-55575

<151> 2002-09-13

<160> 19

<170> KopatentIn 1.71

<210> 1

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 1

<400> 1

ggctttcag ccactcctt ggtgaag

27

<210> 2

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 2

<400> 2

gcggatcctc agggggtgac gat

23

<210> 3

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 3

<400> 3

gcggatccgg gggtgacgat gccggag

27

<210> 4

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> GPD-err primer

<400> 4

gcagagctaa ccaataagg

<210> 5
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T-0 primer

<400> 5
 tgcaattgaa cacaaccac 19

<210> 6
 <211> 1023
 <212> DNA
 <213> Candida antarctica

<220>
 <221> sig_peptide
 <222> (1)..(51)
 <223> secretion signal

<400> 6		
atgaatataat tttacatatt tttgttttg ctgtcattcg ttcaaggtac cgccactccc		60
tttgtaagc gtctgccttc cggttcggac cctgcctttt cgcaagccaa gtcggtgctc		120
gatgcgggtc tgacctgcca gggtgcttcg ccattcctcg tctccaaacc catccttctc		180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct		240
gcmcagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatgct caacgacacc		300
caggtcaaca cggagtagcat ggtcaacgccc atcaccacgc tctacgctgg ttcccggcaac		360
aacaagcttc ccgtgctcac ctggcccag ggtggctgg ttgcacagtgg gggtctgacc		420
ttcttccccca gtatcagggtc caaggtcgat cgacttatgg cctttgcgcc cgactacaag		480
ggcaccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag		540
caaaccaccc gttcggact cactaccgca ctccgaaacg caggtggctt gacccagatc		600
gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac		660
tcgcccactcg actcatccta cctcttcaac gggaaagaacg tccaggcaca ggctgtgtgt		720
gggccgctgt tcgtcatcgaa ccatgcaggc tcgctcacct cgcaattctc ctacgtcg		780
ggtcgatccg ccctgcgcgc caccacgggc caggctcgta gtgcagacta tggcattacc		840
gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggtcgc cgccggctgc		900
ctcccgccgc cggcggctgc agccatcgta gcgggtccaa agcagaactg cgagcccgac		960
ctcatgccct acgcccggccc ctttgcagta ggcaaaagga cctgctccgg catcgtaacc		1020
ccc		1023

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<210> 7
<211> 1023
<212> DNA
<213> *Candida antarctica*

<220>
<221> sig_peptide
<222> (1)..(51)
<223> secretion signal

<400> 7
atgaatatat tttacatatt tttgttttg ctgtcattcg ttcaaggtac cgccactcct 60
ttggtaagc gtctgccttc cggttcggac cctgccttt cgcaagccaa gtcggtgctc 120
gatgcgggtc tgacctgcca aggtgcttcg ccacccctcg tctccaaacc catccttctc 180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct 240
gcccagctgg gttacacacc ctgctggatc tcaccccccgc cggtcatgct caacgacacc 300
caggtcaaca cggagttacat ggtcaacgccc atcaccacgc tctacgctgg ttcgggcaac 360
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggctgacc 420
ttcttccca gtatcaggc caaggcgtat cgttatgg ccttgcgc cgaactacaag 480
ggcaccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag 540
caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600
gtgcccacca ccaacctcta ctcggcgtacc gacgagatcg ttcagcctca ggtgtccaac 660
tcgcccactcg actcatccta cctttcaac ggaaagaacg tccaggcaca ggctgtgtgt 720
ggccgcagt tcgtcatgca ccatgcaggc tcgctcacct cgcaattctc ctacgtcg 780
ggtcgatccg ccctgcgtc caccacgggc caggctcgta gtgcggacta tggcattacg 840
gactgcaacc ctcttccgc caatgatctg actcccggagc aaaaggtcgc cgccgcg 900
ctccggcgc cggcggctgc agccatcgta gcgggtccaa agcagaactg cgagccgac 960
ctcatgcctt acgcccggccc ctttgcgtat ggcaaaaagga cctgctccgg catcgacc 1020
ccc 1023

<210> 8
<211> 1023
<212> DNA
<213> *Candida antarctica*

<220>
<221> sig_peptide
<222> (1)..(51)
<223> secretion signal

<400> 8

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atgaatatat tttacatatt tttgttttg ctgtcattcg ttcaaggtac cgccactcct	60
ttggtaagc gtctgccttc cggtcggac cctgccttt cgcaagccaa gtcggtgctc	120
gatgcgggtc tgacctgcca gggtgctcg ccatcctcg tctccaaacc catcctctc	180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctc	240
gchgagctgg gttacacacc ctgctggatc tcaccccgcc cgttcatgct caacgacacc	300
caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcggcaac	360
aacaagcttc cctgtctcac ctggtcccag ggtggctgg ttgcacagtg gggtctgacc	420
ttctccca gtatcaggc caaggtcgat cgacttatgg ccttgcgcc cgactacaag	480
ggcaccgtcc tcgccggccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag	540
caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggct gacccagatc	600
gtgcccacca ccaacctcta ctcggcacc gacgagatcg ttcaagcctca ggtgtccaac	660
tcgccactcg actcatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt	720
ggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcaaggctc ctacgtcg	780
ggtcgatccg ccctgcgctc caccacggc caggctcgta gtgcagacta tggcattacg	840
gactgcaacc ctctccgc caatgatctg actcccagc aaaaggtcgc cgccgctgc	900
ctcctggcgc cggcggctgc agccatcgagc gcgggtccaa agcagaactg cgagccgac	960
ctcatgcct acgcccggcc ctttgcagta ggcaaaagga cctgctccgg catcgtaacc	1020
ccc	1023

<210> 9
<211> 343
<212> PRT
<213> *Candida antarctica*

<220>
<221> SIGNAL
<222> (1)..(17)
<223> secretion signal

<400> 9
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

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Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340

<210> 10
<211> 343
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (1)..(17)
<223> secretion signal

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<400> 10
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240
Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
290 295 300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

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325

330

335

Gly Ile Val Thr Pro Gly Ser
 340

<210> 11
 <211> 341
 <212> PRT
 <213> Candida antarctica

<220>
 <221> SIGNAL
 <222> (1)..(24)
 <223> secretion signal

<400> 11
 Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 1 5 10 15
 Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
 20 25 30
 Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
 35 40 45
 Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
 50 55 60
 Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
 65 70 75 80
 Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
 85 90 95
 Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
 100 105 110
 Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
 115 120 125
 Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
 130 135 140
 Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
 145 150 155 160
 Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
 165 170 175
 Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
 180 185 190
 Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
 195 200 205
 Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
 210 215 220
 Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
 225 230 235 240

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Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Leu Ala Pro
290 295 300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335
Gly Ile Val Thr Pro
340

<210> 12
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> CALB primer 4

<400> 12
ctcatatgct accttccggt tcggac 26

<210> 13
<211> 21
<212> PRT
<213> Artificial sequence

<220>
<223> a-amylase secretion signal

<400> 13
Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala
1 5 10 15
Ala Pro Ala Leu Ala
20

<210> 14
<211> 317
<212> PRT
<213> Candida antarctica

<400> 14
Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
1 5 10 15
Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

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Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60

Trp Ile Ser Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

<210> 15
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> LQ53 primer

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<400> 15
gctgtgtgtg ggccgcagtt cgtcatcg 28

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LP35 primer

<400> 16
gcatggtcga tgacgaactg cggcccacac 30

<210> 17
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LP53 primer

<400> 17
gtcgccgcgg ctgcgctccc ggcgccggcg 30

<210> 18
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> LP35 primer

<400> 18
ctgcagccgc cggcgccggg agcgcagcc 29

<210> 19
<211> 343
<212> PRT
<213> Candida antarctica

<400> 19
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

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Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
 65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
 85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
 100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
 115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
 130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
 145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
 165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
 180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
 195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
 210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
 225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
 245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
 260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
 275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Leu Ala Pro
 290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
 305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
 325 330 335

Gly Ile Val Thr Pro Gly Ser
 340